REMARKS/ARGUMENTS

Claims 1-62 are pending in the application.

Claims 1-17 and 43-62 stand rejected.

Claim 18-42 have been canceled and such cancellation is without prejudice or waiver.

As a result of canceling claims 18-42, the undersigned is requesting under separate cover a request for inventorship correction under 37 CFR 1.48(b).

THE REJECTION UNDER 35 U.S.C. § 112

Claims 1-14, 6-7, 43-46,48-53 and 54-62 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

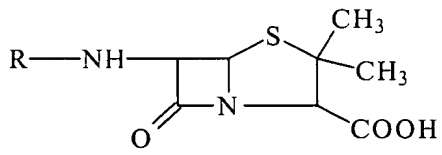
The Examiner states that it is vague and indefinite what is meant by the terms "penicillin-class substrate" and "cephalosporin class substrate".

Applicant has now amended the rejected claims to include the wording "catalyses the hydrolysis of the amide bond of ß-lactam rings in penicillin or cephalosporin compounds." Support for this language is found in page 2, third paragraph which reads as follows:

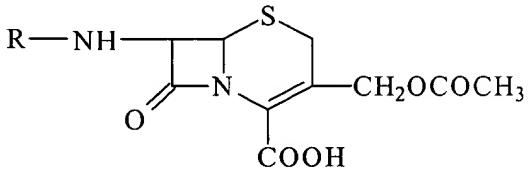
"The TEM-1 ß-lactamase is a member of a family of bacterial enzymes that hydrolyze antibiotics of the penicillin and cephalosporin class, thus imparting resistance to bacteria expressing these enzymes. TEM-1 ß-lactamase is the standard ampicillin resistance gene included in most plasmids used in molecular biology. The three-

dimensional structure, proposed catalytic mechanism and optimal substrates and inhibitors have been well documented. TEM-1 ß-Lactamase is a small (29 kiloDaltons) and monomeric protein consisting of 286 amino acids. The first 23 amino acids constitute a secretory signal peptide. ß-lactamases catalyses the irreversible hydrolysis of the amide bond of ß-lactam rings in penicillin or cephalosporin compounds. ß-lactamases are secreted into the periplasmic space of gram-negative strains or into the outer media by their gram-positive counterparts where they normally act. However, they will accumulate in the cytoplasm when expressed in *E. coli* or other prokaryotic or eukaryotic cells if the secreting signal peptide is genetically deleted, without effecting catalytic activity."

It should be pointed out that penicillin and cephalosporin type antibiotics are very well known molecules in the art and that both types of compounds have ß-lactam rings in their chemical structures. For the Examiners benefit, applicant is presenting the basic chemostructural nucleus of penicillin molecules and cephasloporin molecules.



Penicillin basic nucleus



Cephalosporin basic nucleus

As the Examiner will appreciate, the four membered ring in both molecules is the ß-lactam ring that gets hydrolyzed by ß-lactamase.

By virtue of the current claim amendments, it is believed that the rejection under 35 U.S.C. 112 second paragraph is now moot.

THE REJECTION UNDER 35 U.S.C. § 102 (e)

The rejection of Claims 1-17 and 43-62 under 35 U.S.C. 102(e) as being anticipated by Cornish (US2002/0004202 A1) is respectfully traversed.

In the rejection, the Examiner states that "Cornish teaches the assay method of claims 1, 8, 12, 43, 56, comprising (see abstract):

(A) generating:

1) at least a first fragment of a reporter molecule linked to a first interacting domain and at least a second fragment of a reporter molecule linked to a second interacting domain, (see page 5, paragraphs 81-83, pages 12-14, example 2) or

2) nucleic acid molecules that code for A)1) and subsequently allowing said nucleic acid molecules to produce their coded products (see page 10, paragraph 017 which shows nucleic acid encoding protein chimeras); then,

(B) allowing interaction of said domains (see page 5, paragraph 083 and page 14, paragraph 0214; and

(C)detecting reconstituted reporter molecule activity (see page 5, paragraph 0084 and page 14, paragraphs 0215-0219), where said reporter molecule can react with a penicillin- or cephalosporin-class substrate (see page 14, paragraph 0215, where nitrocefin, cephalosporin-class substrate (see page 14, paragraph –215, where nitrocefin, cephalosporinase substrate is used)."

In the Cornish invention as described in US 2002/0004202, a chimeric molecule (the dimerizer) that contains three components is synthesized: one moiety that is a high-affinity ligand for one protein, one that is a ligand for another protein, and a linker that

contains a substrate for some specific or general class of enzymes. The dimer is introduced into yeast containing a two-hybrid transcriptional reporter assay system consisting of the two proteins to which the dimerizer binds, fused to complementary DNAbinding and RNApolymerase activating domains, respectively. The dimerizer binds to the two proteins simultaneously, allowing for transcription of a reporter gene whose presence can be detected by enzymatic assays. The two dimerizing proteins are dihydrofolate reductase and glucocortocoid receptor ligand-binding domains. These are fused to LexA DNA binding and B42 RNA polymerase activation domains, respectively. In the specific cases presented by Cornish, an assay for detection of an active form of a cephalosporinase of Enterobacter cloacae called P99 is presented. Thus, the dimerizer consists of methotrexate linked via a thioether to the β-lactam cephalosporin and in turn, to dexamethasone by a peptide bond (Mtx-cephem-Dex). When these proteins are expressed in the budding yeast *Saccharomyces cerevisiae* grown on medium containing an appropriate concentration of the dimerizer, simultaneous binding of dihydrofolate reductase-LexA to the Mtx moiety and of glucocortocoid receptor-B42 to the Dex moiety of Mtxcephem- Dex, results in reconstitution of an active LexA promoter and transcription of the β-galactosidase reporter gene. The activity of the β-galactosidase gene product is detected by using substrates (5-bromo-4-chloro-3-indolyl β-D-galactoside and o-nitrophenyl-D-b-galactoside) that are converted to colored or fluorescent products by the β-galactosidase.

In response to the Examiner's rejection, it should be pointed out that <u>*Cornish uses*</u>

a full-length enzyme to demonstrate that lactamase activity can be detected with their "three hybrid" transcriptional reporter assay.

In contradistinction, *applicant uses fragments of the enzyme that have been engineered* to create a protein complementation assay (PCA). As the Examiner will note, the PCA strategy of the present invention is based *on the reassembly of two rationally designed complementary fragments of TEM-1 ß-lactamase*. Cornish is totally silent on the use of enzyme reporter fragments in the assay of the instant invention.

In the present invention, Applicants cuts the *TEM-1 ß-lactamase* in the middle of the loop between residues Glu197 and Leu198 and additionally the secreting signal peptide of 23 amino acids was deleted to leave only the functional enzyme. Accordingly, one creates fragment [1] (BLF[1]) which consists of residues 24 to 197 and fragment [2] (BLF[2])which consists of residues 198-286. Each of these fragments are linked to interacting domains (GCN 4 leucine Zipper or the pair of rapamycin inducible interacting proteins FKBP/FRB domain) by a linker of 15 amino acids (Gly- Gly- Gly- Gly- Ser)3.

In the present invention fragment means an engineered polypeptide fragments(see above), which when brought together in space by interacting proteins to which fragments are fused, fold and regain structure and enzymatic activity.

Cornish on the other hand, refers to activation and DNA binding domains of three hybrid reporter system as "fragments" when they are in fact fully functional and folded binding domains.

Furthermore, *the complementation method of the present invention refers to folding and regain of function of an enzyme from fragments brought together in space by interacting proteins.* In Cornish, they refer to: "A reaction-independent complementation assay for detecting enzyme activities of genes or artificial libraries of genes." Complementation means conferring survival or a color of the cell in which their assay is expressed by virtue of expression of a reporter gene controlled by a three-hybrid system.

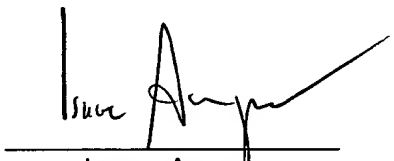
Additionally, in the present invention, an individual PCA enzyme reconstituted from fragments is the "reporter" of a protein-protein interaction. Cornish means a reporter gene expressed under the control of a three-hybrid-regulated promoter.

Regarding the use of the penicillin or cephalosporin substrates applicant uses various fluorescent and colored analogs of these molecules for the beta-lactamase PCA. Cornish creates tripartite molecules consisting of fusions between a cephalosporin and two other moieties that will bind and non-covalently crosslink two proteins.

In summary, it is clear that the assay of the invention using fragments of β-lactamase which when complemented catalyses the hydrolysis of the amide bond of ß-lactam rings in penicillin- or cephalosporin-class compounds is quite different from the assay of Cornish which uses a reporter gene expressed under the control of a three-hybrid-regulated promoter.

In view of the above Amendments and remarks, it is respectfully submitted that the Claims of the current Application are in condition for allowance. Reconsideration and withdrawal of the rejections are requested. The Examiner is invited to contact the undersigned at 703-418-2777 if he feels that further discussion may facilitate the resolution of any outstanding issues. An early indication of a Notice of Allowance is earnestly solicited.

Respectfully submitted,



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